# Haematopoietic gene therapy of non-conditioned patients with Fanconi anaemia-A: results from open-label phase 1/2 (FANCOLEN-1) and long-term clinical trials

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## Summary

**Background** **Allogeneic haematopoietic stem-cell transplantation is the standard treatment for bone marrow failure (BMF) in patients with Fanconi anaemia, but transplantation-associated complications such as an increased incidence of subsequent cancer are frequent. The aim of this study was to evaluate the safety and efficacy of the infusion of autologous gene-corrected haematopoietic stem cells as an alternative therapy for these patients.**

**Methods** **This was an open-label, investigator-initiated phase 1/2 clinical trial (FANCOLEN-1) and long-term follow-up trial (up to 7 years post-treatment) in Spain. Mobilised peripheral blood (PB) CD34⁺ cells from nine patients with Fanconi anaemia-A in the early stages of BMF were transduced with a therapeutic *FANCA*-encoding lentiviral vector and re-infused without any cytotoxic conditioning treatment. The primary efficacy endpoint of FANCOLEN-1 was the engraftment of transduced cells, as defined by the detection of at least 0·1 therapeutic vector copies per nucleated cell of patient bone marrow (BM) or PB at the second year post-infusion, without this percentage having declined substantially over the previous year. The safety coprimary endpoint was adverse events during the 3 years after infusion. The completed open-label phase 1/2 and the ongoing long-term clinical trials are registered with ClinicalTrials.gov, NCT03157804; EudraCT, 2011–006100–12; and NCT04437771, respectively.**

**Findings** **There were eight evaluable treated patients with Fanconi anaemia-A. Patients were recruited between Jan 7, 2016 and April 3, 2019. The primary endpoint was met in five of the eight evaluable patients (62·50%). The median number of therapeutic vector copies per nucleated cell of patient BM and PB at the second year post-infusion was 0·18 (IQR 0·01–0·20) and 0·06 (0·01–0·19), respectively. No genotoxic events related to the gene therapy were observed. Most treatment-emergent adverse events (TEAEs) were non-serious and assessed as not related to therapeutic *FANCA*-encoding lentiviral vector. Nine serious adverse events (grade 3–4) were reported in six patients, one was considered related to medicinal product infusion, and all resolved without sequelae. Cytopenias and viral infections (common childhood illnesses) were the most frequently reported TEAEs.**

**Interpretation** **These results show for the first time that haematopoietic gene therapy without genotoxic conditioning enables sustained engraftment and reversal of BMF progression in patients with Fanconi anaemia.**

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## Introduction

Fanconi anaemia is a rare, predominantly autosomal recessive DNA-repair disorder.[1,2] Bone marrow failure (BMF) is a major disease-related cause of morbidity, occurring in approximately 80% of patients with Fanconi anaemia during the first decade of life.[3,4] Fanconi anaemia is additionally associated with a high incidence of haematological and solid tumours.[3,5] Patients with Fanconi anaemia-A (with mutations in *FANCA*) comprise approximately 60–70% of cases worldwide among the 23 Fanconi anaemia subtypes.[6,7] Allogeneic haematopoietic stem-cell transplantation (HSCT) constitutes the only potentially curative therapy for Fanconi anaemia-related BMF, although it is associated with substantial short-term and long-term toxicities.[8–11] These toxicities include a very high incidence of head and neck squamous cell carcinomas, with carcinogenesis risk likely amplified by genotoxic conditioning and graft-versus-host disease.[5,12] Safer therapies that can correct or prevent Fanconi anaemia-associated BMF are thus needed.

It has been postulated that gene therapy might constitute an alternative to allogeneic HSCT in Fanconi anaemia,[13,14]

## Research in context

### Evidence before this study

We searched PubMed from database inception to June 1, 2024 for publications focused on outcomes of patients with Fanconi anaemia either treated with allogeneic haematopoietic stem cell transplantation (HSCT) or gene therapy. We also searched ClinicalTrials.gov for related clinical trials. Fanconi anaemia is an inherited disease with a very high incidence of bone marrow failure (BMF) and cancer predisposition. Currently, allogeneic HSCT is the only curative therapy for Fanconi anaemia-related BMF, although it is associated with severe short-term and long-term toxicities, including further increased risk of malignancies. Although gene therapy was initially proposed as an ideal alternative to allogeneic HSCT in patients with Fanconi anaemia, none of the early gene therapy trials developed for these patients resulted in successful engraftment of gene corrected cells. More recent clinical studies from our consortium have shown, however, that the use of optimised haematopoietic stem cell (HSC) collection and transduction processes in four patients with Fanconi anaemia subtype A (Fanconi anaemia-A) conferred progressive engraftment of gene-corrected HSCs in the absence of antecedent conditioning in the short term (18–30 months post-infusion).

### Added value of this study

To our knowledge, the results obtained in these phase 1/2 and long-term follow-up clinical trials show for the first time that gene therapy can revert BMF progression in patients with Fanconi anaemia-A. This was achieved in the absence of any cytotoxic conditioning, which enabled almost immediate patient discharge from hospital after cell infusion, and markedly reduced short-term and longer-term toxicity. Although treatments with very low numbers of transduced $CD34^+$ cells did not halt BMF in these patients, two of the three patients infused with more than 240 000 transduced $CD34^+$ cells per kg showed evident correction of BMF progression, an observation that was sustained until the most recent follow-up, up to 7 years post-infusion.

### Implications of all the available evidence

Findings from this clinical study provide evidence of safety and efficacy of a non-toxic gene therapy approach to treat BMF in patients with Fanconi anaemia, a disease characterised by cancer predisposition and hypersensitivity to cytotoxic drugs. Gene therapy approaches similar to the one proposed in this study could potentially be applied to other BMF syndromes.

but previous gene therapy trials had failed to show engraftment of corrected cells in these patients.[15–17] In preclinical studies we first showed that transduction of Fanconi anaemia-A $CD34^+$ cells with a *FANCA*-lentiviral vector (PGK-*FANCA*.Wpre* lentiviral vector) conferred a strong proliferation advantage to these cells following transplantation into immunodeficient mice.[18] More recently, we showed that the combined administration of granulocyte colony-stimulating factor and plerixafor facilitated the collection of $CD34^+$ cells in patients with Fanconi anaemia-A.[19] Based on these experimental and clinical data, a phase 1/2 gene therapy trial (FANCOLEN-1) was initiated in 2016. Preliminary results from this trial showed that infusion of transduced $CD34^+$ cells in four patients with Fanconi anaemia-A who had not received pre-treatment conditioning conferred progressive engraftment of gene-corrected haematopoietic stem cells (HSCs) during the 18–30 months post-infusion, although at that time no evidence of haematological improvement was observed.[20] Herein, we report the complete results of the FANCOLEN-1 clinical trial, with a planned duration of 3 years, and the long-term follow-up trial. The longest follow-up of these patients was 7 years post-infusion and the median follow-up was 3 years (IQR 2–7).

## Methods

### Study design and participants

FANCOLEN-1 is an open-label, investigator-initiated phase 1/2 clinical trial (ClinicalTrials.gov, NCT03157804; EudraCT, 2011–006100–12) involving patients with Fanconi anaemia-A treated with lentiviral-mediated gene therapy. Patients were treated after written informed consent was provided by parent(s) or guardian(s). For $CD34^+$ cell collection, patients had to be in early stages of BMF as defined in the FANCOSTEM-1 clinical trial (EudraCT, 2011–006197–88; ClinicalTrials.gov, NCT02931071; ECT 2011–006197–88).[19] Therefore, at least one of the following parameters had to exceed these values: haemoglobin: 8·0 g/dL; neutrophils: 750/mm³; platelets: 30·000/mm³. For the infusion of transduced cells, the FANCOLEN-1 trial inclusion criteria initially required that patients have some evidence of BMF (haemoglobin <8 g/dL; neutrophils <750 cells per mm³; or platelets <30 000 cells per mm³; see version 4 of the FANCOLEN-1 protocol in the appendix p 31). In 2018, a substantial protocol amendment was approved, which eliminated this criterion (see version 5 of FANCOLEN-1 in the appendix p 31). In all instances, transduced $CD34^+$ cell grafts were infused without cryopreservation, aiming at preventing previously described cryopreservation-associated cell losses.[19]

An Independent Ethics Committee (IEC) of Hospital Infantil Universitario Niño Jesús of 13 independent experts (paediatricians, pharmacist, surgeon, clinical analysis specialists, psychiatrist, anaesthetists, nurse, and a lawyer with previous experience in evaluating research project methodology and legal and ethical aspects) reviewed all adverse events reported during the trial. Adverse events were routinely reported within 7 days of occurrence, except for serious adverse events which were reported within 24 h. Adverse events were reviewed periodically (at least once every 3 months) by a

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See **Online** for appendix

steering committee, and at least annually by the IEC. MRa, a trial investigator and IEC member, recused himself from the committee's discussions regarding the clinical trial in accordance with the committee's working rules.

The trial was conducted at Hospital Infantil Universitario Niño Jesús (Madrid) and Hospital Vall d'Hebron (Barcelona). FANCOLEN-1 was sponsored by Fundación para la Investigación Biomédica del Hospital Infantil Universitario Niño Jesús. On July 15, 2016, Rocket Pharmaceuticals (Rocket Pharma) was granted a licence for further development of gene therapy for Fanconi anaemia-A, and sponsored the long-term follow-up trial to follow up patients for up to 15 years post-infusion (ClinicalTrials.gov, NCT04437771). Both trials were conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. The study protocols and all amendments were reviewed by the Clinical Research and Ethics Committee from each centre (appendix p 28), and authorised by the Spanish Agency of Medicines and Medical Devices. The Instituto de Salud Carlos III and the European Commission funded FANCOLEN-1, and Rocket Pharma sponsored the long-term follow-up trial. Efficacy results from patients who did not receive treatment with eltrombopag or allogeneic HSCT due to BMF progression were censored at time of these therapies.

## Procedures

The data cutoff date was June 27, 2023. CD34$^+$ cells from patients with Fanconi anaemia were mobilised with filgrastim and plerixafor as described in the FANCOSTEM clinical trial (ClinicalTrials.gov, NCT02931071; EudraCT: 2011–006197–88), which aimed to evaluate the safety and efficacy of an HSC mobilisation regimen in patients with Fanconi anaemia for subsequent use in gene therapy.[19] Freshly collected or cryopreserved CD34$^+$ cells were transduced with the PGK-*FANCA*.Wpre* lentiviral vector[20] and infused intravenously immediately into patients without any antecedent conditioning treatment (ie, no chemotherapy or radiation was administered). In five patients (patients 2, 3, 5, 6, and 8), CD34$^+$ cells were cryopreserved until peripheral blood (PB) cell counts decreased below the threshold defined in the inclusion criteria. In three cases, cells were transduced immediately after collection (patients 1, 4, and 7), and in one case (patient 9) the transduced graft consisted of both fresh and cryopreserved CD34$^+$ cells (table 1). After the protocol amendment was approved in 2018, collected CD34$^+$ cells were not cryopreserved, thus preventing cryopreservation-associated cell losses that were previously described.[19]

## Outcomes

The primary efficacy endpoint of the FANCOLEN-1 study was the engraftment of transduced cells, as defined by the detection of at least 0·1 therapeutic vector copies per nucleated cell of patient bone marrow (BM) or PB at 2 years post-infusion, without this percentage having declined substantially over the previous year. The secondary endpoint was the achievement of a substantial increase in at least one haematological parameter at the end of the second year post-infusion, without this

| | Gender | Race or ethnic group | Age at time of gene therapy (years) | FANCA gene variant | Product | VCN/cell | MMC resistance (%) | CD34$^+$ cell dose/kg | cCD34$^+$ cell dose/kg* |
|---|---|---|---|---|---|---|---|---|---|
| Patient 1 | Male | Romani people and Travellers | 4 | C295C>T; C295C>T | Fresh | 0·17† | 12% | 1 400 000 | 238 000 |
| Patient 2 | Male | Romani people and Travellers | 5 | C295C>T; C295C>T | Cryopr | 0·45† | 30% | 540 000 | 243 000 |
| Patient 3 | Male | White | 7 | C. 1115–1118 Del TTGG; C. 1115–1118 Del TTGG | Cryopr | 0·24‡ | 27% | 708 000 | 169 920 |
| Patient 4 | Male | White | 5 | C. 3788–3790 Del TCT; C. 2851C>G | Fresh | 0·53† | 49%§ | 771 000 | 408 630 |
| Patient 5 | Male | Romani people and Travellers | 6 | C295C>T; C295C>T | Cryopr | ND | ND | 300 000 | .. |
| Patient 6 | Female | White | 7 | C. 4130 C>G; C. 1115–1118 Del TTGG | Cryopr | 0·67‡ | 34% | 72 500 | 48 575 |
| Patient 7 | Male | Arab | 3 | Del EX 1–43; C. 3788_3790 Del TCT | Fresh | 0·71‡ | 77% | 203 000 | 144 130 |
| Patient 8 | Male | White | 6 | C. 1858_1859 insC; C. 893+2 T>C | Cryopr | 0·34‡ | 30% | 395 000 | 134 300 |
| Patient 9 | Female | White | 3 | Del EX 1–43; C. 1115–1118 Del TTGG | Fresh and cryopr | 0·91‡ | 68% | 1 910 000 | 1 738 100 |

VCN/cell was VCN per cell in pooled (†) or individual (‡) colonies after 14 days of growth in semisolid cultures. MMC resistance was percentage of survival of colonies exposed to 10 nM MMC. Cryopr=cryopreserved. MMC=mitomycin C. ND=not determined. VCN=vector copy number. *cCD34$^+$ cell dose/kg corresponds to CD34$^+$ cell dose/kg multiplied by the VCN/cell in pooled (†) or individual (‡) colonies after 14 days of growth in semisolid cultures (no MMC). §Only analyses at 3 nM MMC could be performed in this sample.

***Table 1:*** **Characteristics of the patients and gene therapy medicinal products**

parameter having decreased substantially over the previous year (appendix p 27). Substantial reductions were defined as those of at least 25%.

The safety coprimary endpoint was the evaluation of all adverse events occurring during the 3 years after infusion of transduced cells. Assessments were the following: incidence of adverse events and serious adverse events, and ongoing evaluation of the short-term and long-term safety of the infusion of medicinal product, as assessed by insertion site analysis, replication competent lentivirus, laboratory tests, vital signs, and physical examinations. Treatment-emergent adverse events refer to adverse events and serious adverse events that occurred after infusion of the medicinal product, but not necessarily related to the gene therapy.

As exploratory studies, insertion site analyses of the therapeutic proviruses and vector copy number (VCN) determinations in purified PB and BM populations were also conducted to confirm gene marking in pluripotent stem and progenitor cells. Sonication-based insertion site analysis technology was used to quantify individual integrations within the gene-corrected cell pool. Clonal dominance was defined in this study as a unique integration site contributing more than 10%. In cases where the VCN is less than one copy per cell, a clone can exhibit prominence solely within the gene-modified cell population without exerting dominance over the entire haematopoietic system. Consequently, to account for this, the raw integration site contribution was normalised by the VCN.

To evaluate if the insertion of the therapeutic provirus was associated with phenotypic correction of BM and PB cells, analyses of mitomycin C (MMC) hypersensitivity in BM progenitors, and studies of chromosomal breaks induced by diepoxybutane on PB T cells were periodically evaluated. Enzyme-linked immuno-Spots were conducted in PB samples to evaluate potential immune responses against G protein-vesicular stomatitis virus (VSV-G) and FANCA. Finally, to evaluate potential contribution of the therapy (*vs* underlying Fanconi anaemia) to clonal expansion, comparative genomic hybridisation in BM samples and fluorescence in situ hybridisation analyses in haematopoietic colonies from patient 1 were performed.

### Statistical analysis

The analyses for FANCOLEN-1 and the long-term follow-up trial were done in the modified intention-to-treat population. The sample size for this first trial was determined based on one that would enable initial understanding of safety and potential efficacy of gene therapy in paediatric patients with Fanconi anaemia, and to enable generation of additional hypotheses to be evaluated in subsequent studies. The ongoing global phase 2 studies (NCT04248439) were subsequently designed to test specific efficacy hypotheses in a more homogeneous Fanconi anaemia-A population, based on learnings from the current study. Anderson–Darlin, D'Agostino and Pearson, Shapiro–Wilk, and Kolmogórov–Smirnov normality tests were applied for testing normality of the distribution in the different parameters analysed: samples following a normal distribution were analysed by unpaired *t* test and represented as mean (SD) and variables following non-normal distribution were reported as median (IQR). Statistical analyses were performed using the GraphPad Prism software package for Windows (version 9; GraphPad Software). Multilevel correlation analysis was performed to analyse the correlation of PB or BM VCN/cell with BM MMC resistance using the correlation package[21] in R (version 4.0.5) and RStudio (version 1.4.1106). An ANCOVA regression analysis was conducted to assess the relationship between baseline factors and endpoints of interest at year 2. The analysis was conducted using R version 4.4.1 and validated by SAS Life Science Analytics Framework 5.3.1.

### Role of the funding source

Authors from Rocket Pharma contributed to the long-term follow-up trial design, data review, and writing of the article. Other funding sources did not have any additional role in the study. The sponsor institutions of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

Nine patients (seven male and two female) with a median age of 5 years (IQR 3·5–6·5) were enrolled at Hospital Infantil Universitario Niño Jesús (table 1, figure 1). Patients were recruited between Jan 7, 2016 and April 3, 2019. All patients met the clinical protocol inclusion criteria, which included a maximum age of 21 years, and did not meet any of the exclusion criteria (appendix pp 25–26). Patients had confirmed mutations in *FANCA* (Fanconi anaemia subtype A; table 1) with demonstrated hypersensitivity of BM colony forming cells to the DNA-damaging agents MMC and PB T cells to diepoxybutane (not shown). In eight of the nine patients, the manufacturing products met the protocol-defined early release specifications. In one case (patient 5), the product showed contamination with *Staphylococcus epidermidis*, hampering the analysis of the graft specifications, and this patient was considered non-evaluable for efficacy.

The characteristics of transduced grafts are shown in table 1. VCN/cell in haematopoietic colonies generated from the manufacturing product showed a median value of 0·49 (IQR 0·27–0·70) copies/cell, indicating that most of the corrected progenitors were likely to harbour 1 copy of the therapeutic provirus. Cryopreserved cells showed no differences in their susceptibility to transduction compared with fresh cells (mean 0·43 [SD 0·18] and 0·47 [0·28] copies/cell, respectively; p=0·80) nor in their MMC resistance (mean 30·30% [SD 2·87%] and mean 45·93% [32·32%], respectively; p=0·37) after the

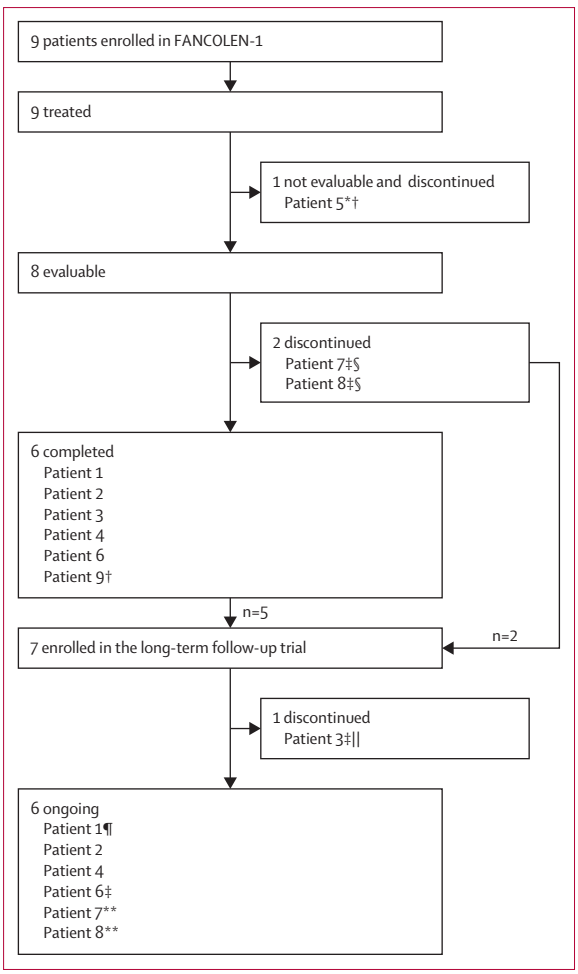


***Figure 1:*** **Trial profiles for the FANCOLEN-1 and long-term follow-up clinical trials**

HSCT=haematopoietic stem cell transplantation. *Not evaluable for efficacy due to contamination of the infusion product with *Staphylococcus epidermidis*, impeding the analysis of the graft specifications. †Did not enrol on long-term follow-up. ‡Received eltrombopag. §Received allogeneic HSCT. ¶Received transfusions. ||Deceased after allogeneic HSCT. **Patients 7 and 8 received allogeneic transplant and were then included in the long-term follow-up trial. Patient 3 died due to complications after allogeneic HSCT.

transduction process. The estimated median number of corrected CD34⁺ cells per kg infused was 203 960 (IQR 136 758–367 223).

The kinetics of gene marking in PB cells from the eight evaluable patients are shown in figure 2A. Apart from patient 6, who was infused with the lowest number of corrected CD34⁺ cells (48 575 corrected CD34⁺ cells per kg), and patient 9, who was infused with a relatively high number of both fresh and cryopreserved cells (1·738 million corrected CD34⁺ cells per kg), the remaining six patients showed progressive increases of gene marking in PB cells.

Two of the three patients who received at least 240 000 corrected CD34⁺ cells per kg (patients 2 and 4) achieved near-complete engraftment levels persisting up to 7 years post-infusion. Gene marking levels determined in BM (figure 2B) were highly consistent with those evaluated in PB, and collectively indicate that the primary efficacy endpoint of FANCOLEN-1 was reached in five of eight evaluable patients. Exploratory studies of VCNs in purified PB and BM subpopulations confirmed that gene marking was evident in all tested lineages, including myeloid, lymphoid, megakaryocytic, erythrocytic, and even in undifferentiated progenitor cells (appendix p 10), strongly suggesting the transduction and engraftment of multipotent HSCs.

Samples from the six patients with at least 0·02 copies per cell in PB or BM were studied by insertion site analyses. Consistent with the reduced number of infused CD34⁺ cells and the absence of conditioning, relatively low numbers of repopulating clones (each represented by a unique insertion site; UIS) were observed (depicted at the bottom of each table in the appendix pp 11–16). In most patients, insertions near proto-oncogenes remained very low and were predominantly transient (appendix pp 11–16). Integrations showed VCN-normalised contributions above 10% in only one patient. At the most recent evaluated timepoint in patient 2, one integration in the *ENG* gene (encoding for the cell surface protein endoglin and predominantly expressed on endothelial cells) was observed at levels that reached 16% (18/110 UISs at month 84). Additionally, a good correlation between the VCN/cell and the total number of UISs was observed (appendix p 17), suggesting that HSC clonal abundance accounted for high engraftment levels.

Insertion site analyses also enabled the follow-up of the HSC clonal reconstitution kinetics (appendix pp 18–20). As shown by the HSC clonal dynamics and the Gini, Shannon, and UC50 (unique cell progenitors contributing the most expanded 50% of progeny cell clones) parameters, patients with the highest levels of reconstitution (patients 2 and 4) exhibited a prominent polyclonal reconstitution (appendix pp 18–19). Additionally, the Venn diagrams representing the UISs common to different lympho-haematopoietic lineages demonstrated the presence of gene-corrected multipotent HSC clones. As expected, a more reduced clonal repertoire and fewer numbers of multipotent precursors were seen in patients with lower levels of engraftment (appendix pp 18–20).

Functional studies showed progressive decreases in the proportion of T cells with chromosomal aberrations in patients with higher VCNs, which in some instances reached levels characteristic of healthy donors (appendix p 21). Furthermore, progressive increases in the proportion of the MMC-resistant colony forming cells were observed, which in the case of patient 2 was in the range corresponding to healthy donor colony forming cells (appendix p 21). Importantly, there was a strong correlation between VCNs determined in either PB or BM with colony forming cell MMC-resistance values, confirming that the phenotypic correction (resistance to

DNA-damage) of haematopoietic progenitors was due to the integration of the therapeutic provirus in these cells (appendix p 21), and subsequently resulted in the repopulation of the BM and PB with gene-corrected cells.

To assess the efficacy of gene therapy to correct BMF, PB cell parameters were evaluated regularly. Two of the three patients who were infused with more than 240 000 corrected CD34⁺ cells per kg (patients 2 and 4), and who showed the highest levels of engraftment, demonstrated evident correction of BMF progression (appendix p 22). Haematological improvements in these two patients were evident in both the erythroid and platelet lineages at 3 years post-infusion, with concomitant neutrophil stabilisation; these were sustained through the latest follow-up, up to 7 years after treatment. In the case of non-engrafted patients or patients with more limited or delayed engraftment, BMF progression was not reverted (appendix p 22), and required salvage therapies, such as investigational eltrombopag or allogeneic HSCT (or both; see details in the appendix [p 3] and figure 1). This was the case for patients infused with the lowest numbers of corrected CD34⁺ cells and also patient 9, who was the only patient treated with a relatively high number of both fresh and cryopreserved cells (1·738 million corrected CD34⁺ cells per kg). To identify potential associations between baseline parameters and haematological improvement, exploratory multivariate analyses were conducted but did not indicate statistically significant correlation. No T-cell-mediated immune responses against FANCA or VSV-G were observed in this patient nor in any other evaluated patient, as deduced from an interferon-γ enzyme-linked immune absorbent spot (not shown).

Regarding the safety co-primary endpoint, most of the adverse events were related to HSC mobilisation and collection (see details of the FANCOSTEM-1 trial[19]). The absence of conditioning before medicinal product infusion markedly limited the incidence of serious adverse events relative to other gene therapy trials. Several medicinal product-related adverse events were observed during the initial hours after medicinal product infusion. Patient 5 experienced pyrexia which resolved within 24 h, and exacerbation of baseline neutropenia which resolved within 1 month. Patient 8 had worsened anaemia and neutropenia, which also resolved to baseline levels after several weeks. Finally, patient 7 experienced a serious adverse event of *Staphylococcus warnierii* bacteraemia which resolved after antibiotic treatment and without sequelae. All treatment-emergent serious adverse events (TEAEs) and the most frequent TEAEs are recorded in table 2. Cytopenias (frequently observed in patients with underlying BMF syndromes) and viral infections (common childhood illnesses) were the most frequently reported TEAEs and are frequently observed in paediatric patients with an underlying BMF syndrome; these were largely not considered to have resulted from the investigational therapy.

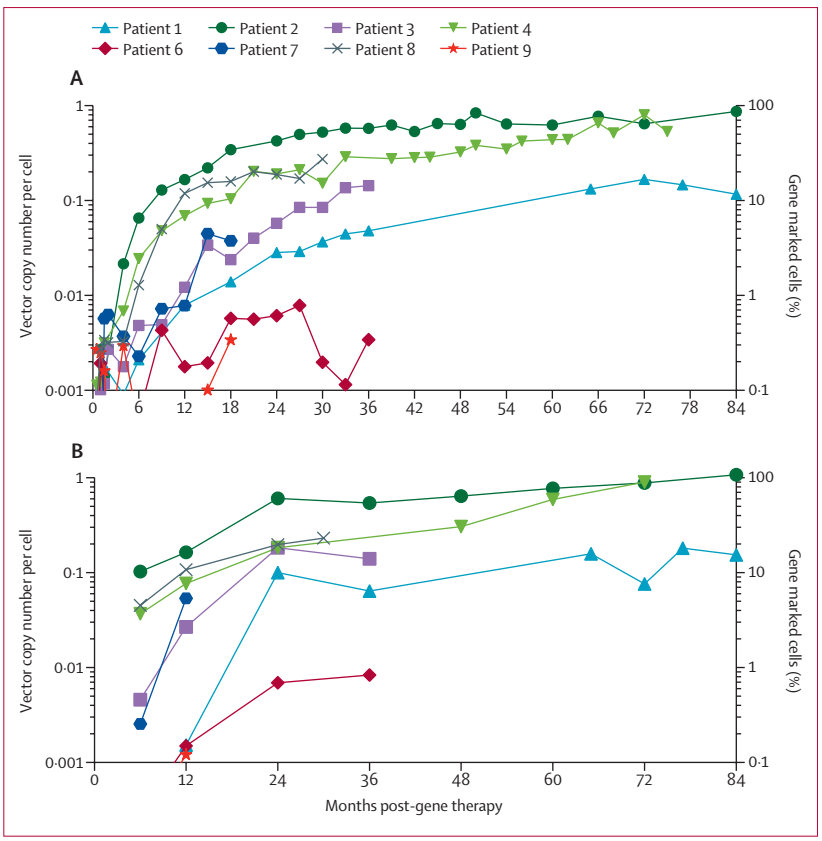


***Figure 2:*** **Engraftment of gene-corrected haematopoietic cells in non-conditioned patients with Fanconi anaemia-A treated with gene therapy**

The figure shows levels of gene marking in peripheral blood (A) and bone marrow cells (B) from patients with Fanconi anaemia-A at different timepoints after infusion of transduced CD34⁺ cells. No detectable vector copies were obtained in analyses of patient 9 from 24 to 36 months post-gene therapy.

A cytogenetic abnormality consisting of a 1q-gain was identified in 5–23% total and purified CD34⁺ BM cells from patient 1 at 72–84 months after gene therapy, when the patient was 10 years old (appendix p 23). To investigate the potential association of the therapeutic provirus with this cytogenetic abnormality, BM samples were cultured in methylcellulose in the absence and the presence of 10 nM MMC. Although 20–63% of the colonies grown in the absence of MMC harboured the 1q-gain at the respective months post-infusion, none of the MMC-selected colonies harboured this cytogenetic abnormality. All tested colonies grown in the presence of MMC contained the therapeutic provirus, showing that the development of the 1q-gain in this patient occurred in uncorrected cells and thus was not due to the insertion of the therapeutic provirus (appendix p 24).

## Discussion

To our knowledge, these results show for the first time that lentiviral-mediated gene therapy can sustainably correct BMF progression in patients with Fanconi

| | Severity | | | | | | | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grade 1 | | Grade 2 | | Grade 3 | | Grade 4 | | Grade 5 | | Serious | | All | |
| | Event(s) | Patient(s) | Event(s) | Patient(s) | Event(s) | Patient(s) | Event(s) | Patient(s) | Event(s) | Patient(s) | Event(s) (n=18) | Patient(s) (n=6*) | Event(s) (n=144) | Patient(s) (n=9) |
| Upper respiratory tract infection | 10 | 3 | 4 | 4 | .. | .. | .. | .. | .. | .. | 1 (6%) | 1 (17%) | 14 (10%) | 7 (78%) |
| Pyrexia | 5 | 2 | 2 | 2 | 3 | 3 | .. | .. | .. | .. | 3 (17%) | 1 (17%) | 10 (7%) | 7 (78%) |
| Neutropenia | .. | .. | .. | .. | 2 | 1 | 6 | 4 | .. | .. | .. | .. | 8 (6%) | 5 (56%) |
| Thrombocytopenia | .. | .. | .. | .. | 2 | 1 | 15 | 4 | .. | .. | .. | .. | 17 (12%) | 4 (44%) |
| Anaemia | 1 | 1 | 1 | 1 | 4 | 1 | 3 | 3 | .. | .. | .. | .. | 9 (6%) | 4 (44%) |
| Gastroenteritis† | 2 | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | 1 (6%) | 1 (17%) | 3 (2%) | 3 (33%) |
| Nasopharyngitis | 8 | 1 | 4 | 2 | .. | .. | .. | .. | .. | .. | .. | .. | 12 (8%) | 3 (33%) |
| Diarrhoea | 2 | 1 | 2 | 2 | .. | .. | .. | .. | .. | .. | 1 (6%) | 1 (17%) | 4 (3%) | 2 (22%) |
| Febrile neutropenia | .. | .. | .. | .. | 1 | .. | 1 | .. | .. | .. | 2 (11%) | 1 (17%) | 2 (1%) | 1 (11%) |
| Osteomyelitis acute | .. | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | 1 (6%) | 1 (17%) | 1 (1%) | 1 (11%) |
| Staphylococcal bacteraemia | .. | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | 1 (6%) | 1 (17%) | 1 (1%) | 1 (11%) |
| Oesophageal food impaction | .. | .. | .. | .. | .. | .. | 1 | .. | .. | .. | 1 (6%) | 1 (17%) | 1 (1%) | 1 (11%) |
| Myositis | .. | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | 1 (6%) | 1 (17%) | 1 (1%) | 1 (11%) |

Data are n (%). Treatment-emergent adverse events following gene therapy administration regardless of causality or attribution were reported during the phase 1/2 study. The long-term follow-up study reports treatment-emergent adverse events that are serious or assessed as related to study drug. *Of the total nine patients treated, six experienced serious adverse events. There were four patients who experienced more than one serious adverse event. †Includes preferred term Campylobacter gastroenteritis.

***Table 2:*** **All serious treatment-emergent adverse events and frequent treatment-emergent adverse events (≥5% of total) by preferred term and severity from phase 1/2 and long-term follow-up studies**

anaemia, the most prevalent inherited BMF syndrome. The ages of the participants in this trial (3–7 years) are within the age range at which a high proportion of patients with Fanconi anaemia begin to develop BMF,[3] but might still have enough HSC reserves to enable mobilisation of sufficient CD34+ cells for successful haematopoietic gene therapy.[19] Progressive increases in the gene marking and phenotypic correction of haematopoietic progenitors and T-lymphocytes were found in patients treated with gene therapy in the absence of cytotoxic conditioning, with stabilisation and improvement of cytopenia conferred by adequate corrected CD34+ cell doses. The absence of any conditioning in this gene therapy approach is of utmost importance in a disease characterised by DNA repair defects and cancer predisposition. The increased cancer incidence observed in patients with Fanconi anaemia after allogeneic HSCT[5,8] is believed to result from cytotoxic conditioning and graft-versus-host disease, and is unlikely to be observed after autologous gene therapy (ongoing long-term follow-up studies will enable assessment of this hypothesis). Additionally, and in contrast to allogeneic HSCT, patients with Fanconi anaemia receiving gene therapy were discharged from hospital within several days following cell infusion.

Despite the absence of conditioning, six of eight evaluable patients showed progressive engraftment of corrected haematopoietic cells, which in some cases reached almost full reconstitution for at least 7 years post-infusion. Analyses of VCN and insertion site analyses in lymphoid and myeloid populations demonstrate for the first time, to the best of our knowledge, that infusion of transduced HSCs in non-conditioned patients enables engraftment of corrected multipotent HSCs and subsequent long-term multi-lineage haematopoietic reconstitution. Additionally, the engraftment of corrected cells was associated with preserved clonal diversity, strongly suggesting the absence of HSC exhaustion, and thus a healthy HSC phenotype, consistent with the normalised transcriptional programme identified in Fanconi anaemia HSCs subsequent to gene therapy.[22]

Although the UIS numbers determined in this Fanconi anaemia trial are markedly lower than those observed in trials for other monogenic disorders, such as β-thalassaemia, sickle cell disease, or different primary immunodeficiencies, this constitutes an expected observation for two main reasons. First, Fanconi anaemia is an HSC defect, so the number of CD34+ cells available for collection and ultimately for infusion were markedly lower compared with diseases in which the HSC phenotype is not affected.[23] Second, because no conditioning was used in our design, corrected HSCs must home and engraft in haematopoietic niches not depleted of endogenous HSCs. Thus, engraftment of corrected HSCs was anticipated to occur much more slowly relative to gene therapy studies that employ cytoreductive treatments.

The reconstitution kinetics of gene-corrected cells observed in this gene therapy trial resemble the evolution of reverted haematopoietic cells in patients with Fanconi

anaemia with somatic mosaicism.[24,25] Compared with non-mosaic patients, reduced incidences of BMF and leukaemia are characteristic of these patients, particularly in patients with multi-lineage mosaicism.[25,26] Although 1q-gain—which has been frequently reported in clonal haematopoiesis in Fanconi anaemia[4]—was identified i n uncorrected haematopoietic progenitors from one of our patients, no myelodysplasias or leukaemias have been observed so far in any patients treated in this trial. It is likely that more rapid and robust engraftment resulting from the infusion of higher numbers of transduced CD34$^+$ cells would limit the expansion of abnormal, potentially preleukaemic clones generated from uncorrected cells, as seen in patients with multi-lineage somatic mosaicism.[25]

Of particular relevance is the statistically significant correlation between levels of gene-corrected cells (as determined by VCN/cell) in PB or BM and the MMC resistance of BM progenitor cells, regardless of whether fresh or cryopreserved cells were infused. This confirms the therapeutic efficacy of the integrated provirus, and also shows that the identification of the therapeutic provirus in PB cells is indicative of MMC resistance in BM progenitor cells; which constitutes the primary biological endpoint of the phase 2 trial.

Limitations of the study include the modest number of patients, given the low prevalence of Fanconi anaemia-A; however, it is notable that two of three patients infused with at least 240 000 corrected CD34$^+$ cells per kg (patients 2 and 4) showed evident correction of BMF progression up to 7 years post-infusion. For comparison, the median number of CD34$^+$ cells infused in these patients represents less than 10% of the corresponding numbers infused in gene therapies of other monogenic diseases,[23] providing further evidence of the extensive repopulating potential of gene-corrected Fanconi anaemia HSCs. At the time of manuscript finalisation, and after the data analysis cutoff d ate, these patients neither received nor required any haematological support, showing the curative potential of gene therapy in BMF. The relevance of infusing threshold numbers of corrected CD34$^+$ cells to arrest BMF progression emphasises the importance of conducting gene therapy during the early stages of Fanconi anaemia-related BMF. The absence of conditioning and very short hospitalisation provide compelling rationale for Fanconi anaemia gene therapy in the early stages of the disease before BMF progression and the need for salvage therapeutic interventions. Based on this study, a global phase 2 trial has been initiated which aims to more precisely delineate the efficacy and safety of gene therapy in a larger cohort of patients with Fanconi anaemia treated in the early stages of BMF, before the onset of severe cytopenias with the goal of averting progression to severe BMF and need for HSCT (NCT04248439).[27]

Additional advantages of gene therapy in Fanconi anaemia could include the haematopoietic tolerance to antineoplastic agents potentially required for treating solid tumours, such as squamous cell carcinoma, which might frequently arise in patients with Fanconi anaemia in subsequent decades.[28]

Altogether, the results from this phase 1/2 and interim analyses of the long-term follow-up trial show that ex vivo gene therapy in the absence of cytotoxic conditioning could revert the natural progression of BMF in patients with Fanconi anaemia and provide a rationale for the development of low-toxicity autologous therapeutic approaches for the treatment of Fanconi anaemia and other inherited BMF syndromes.

#### Contributors

JAB, PR, and JSe were involved in the design of the FANCOLEN-1 clinical trial, as well as in the conceptualisation, data curation, formal analysis, investigation, methodology, validation, funding acquisition, and writing and review of the manuscript; JZ and SN were involved in investigation, methodology, and review; EG, RS-D, ES, MRo, RP, MB, PJ-N, ALB, AS, WW, MS, LL, JCS, RMY, OA, BD, MF-G, LG-G, MRa, RS-D, FL, MC, TL, NGdA, RL-A, AC, JB, SR-P, GR, JSo, and CD-d-H were involved in the investigation and review; EN was involved in the formal analysis, validation, and review; AG was involved in the resources and review; JSu was involved in the investigation, supervision, and review; JDS was involved in the conceptualisation, formal analysis, writing, and review. All authors had access to all the included data and were involved in the collection and interpretation of the data, participated in manuscript review, and accept responsibility to submit the manuscript for publication. PR, EN, and JSe accessed and verified the raw data.

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#### Declaration of interests

PR: has received honoraria as consultant and holds stock options and royalties for licences to Rocket Pharmaceuticals. SN: has received honoraria as consultant and holds stock options and royalties for licences to Rocket Pharmaceuticals. EN: employee of Rocket Pharmaceuticals and owns Rocket Pharmaceuticals equity and equity options. JCS: has received honoraria as consultant and holds stock options and royalties for licences to Rocket Pharmaceuticals. JSu: has received service honoraria from Rocket Pharmaceuticals. GR: employee of Rocket Pharmaceuticals and owns Rocket Pharmaceuticals equity and equity options. JSo: has received honoraria as consultant from Rocket Pharmaceuticals. JDS: employee and officer of Rocket Pharmaceuticals and owns Rocket Pharmaceuticals equity and equity options. JSe: has received support for attending meetings and honoraria as consultant, as member of advisory boards, and holds stock options and royalties for licences to Rocket Pharmaceuticals. JAB: has received honoraria as consultant and holds stock options and royalties for licences to Rocket Pharmaceuticals. AdC: has received honoraria as consultant and holds stock options and royalties for licences to Rocket Pharmaceuticals. The institutions of PR, JZ, SN, AS, PJ-N, ALB, MRa, JCS, JSu, JSe, JAB, and AdC have received funding for research on gene therapy from Rocket Pharmaceuticals. All other authors declare no competing interests.

#### Data sharing

All relevant data collected for the study, including individual participant data, are included in the paper. All individual participant data are de-identified to protect the privacy of study participants. Patient-related

data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality. Additional supporting data and documents such as the study protocol, statistical analysis plan, and informed consent forms are available from the principal investigator of the clinical trial upon request. All requests for raw and analysed data and materials will be reviewed by the principal investigator of the clinical trial at julian.sevilla@salud.madrid.org.

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